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The P-protein in *Ruta chalepensis* L.

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Riassunto

In questo lavoro abbiamo studiato le caratteristiche della P-protein in *Ruta chalepensis* L.

Abbiamo osservato i vari tipi di P-protein già definita come nascente, tubulare e cristallina, inoltre abbiamo notato la P-protein in relazione con i microtubuli, con le spiny vescicole e con i dittiososmi.

INTRODUCTION

Many studies on the P-protein in sieve tubes have been published. P-protein bodies consist of polymorphic aggregates showing different patterns of aggregation and extension in different species; they appear to be tubular in *Nicotiana* (CRONSHAW and ESAU, 1967) and *Coleus* (STEER and NEWCOMB, 1969), granular in *Cucurbita* (CRONSHAW and ESAU, 1968b), fibrillar in *Ricinus* (CRONSHAW, 1975), paracrystalline in certain legumes (WERGIN and NEWCOMB, 1970; ESAU, 1978).

PARTHASARATHY (1975) described various types of P-protein bodies within a single cell. Recently, FJELL (1987) studied the formation of P-protein in *Salix viminalis.*

The morphological changes of P-protein depend on differences in environmental conditions within the cell (CRONSHAW *et al.,* 1973). In a previous study we described sieve-element plastids and their ontogeny in some Rutaceae (MATARESE PALMIERI and TOMASELLO, 1983-1984).

Key words: P-protein, Sieve-element, Rutaceae.

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In this paper, we report the characters of P-protein in *Ruta chalepensis,* with the purpose of studying the morphological changes during their ontogenesis.

Materials and methods

Excised leaves from first and second internodes of *Ruta chalepensis* L. were collected from plants growing in the Botanical Gardens of Messina, Sicily, Italy.

The samples were fixed in cold glutaraldehyde 4%, 6% buffered with 0,2M phosphate buffer at pH 7,2 for 2h, 4h, postfixed in 1% osmium tetroxide in a 0,2M phosphate buffer at pH 7,2 for 2h at 4°C, dehydrated in ethanol and finally embedded in Epon-araldite mixture (Mollenhauer, 1964).

The pieces were then sectioned with and Ultrotome LKB microtome. The sections were stained with uranyl acetate and lead citrate (Reynolds, 1963) and observed with a Siemens Elmiskop 102 A electron microscope.

Observations

Various forms of P-protein bodies were observed in differentiating sieve-elements during the stages of development of leaves in *R. chalepensis.* Plastids, endoplasmic reticulum (ER), mitochondria dictyosomes, nucleus, vacuoles and principally granular P-protein are present in immature sieve-elements (Fig. 1).

During the early stages of development, we observed the disaggregation of endoplasmic reticulum; in the cytoplasm, two forms of P-protein occur tubular and nascent (Fig. 3).

Also, in longitudinal and trasverse sections, both the P-protein of tubular type (Fig. 4) and crystalline type (Fig. 2) were found.

In mature sieve-elements, where either stacks of ER in the shape of circular profiles (Fig. 7, 9) or single parietal layer appear, the P-protein is granular associated with microtubules. In the same tube the P-protein of fibrillar type occurs (Fig. 5). Fig. 2 shows coated and spiny vesicles. The companion cells show protein bodies (Fig. 6), coated vesicles with electrondense material,

dictyosomes, nucleus, a few vacuoles and many ribosomes (Fig. 6). In the sieve plates fine filaments occur.

Fig. 7 and 10 show stacks of ER in the proximity of the cell wall with circular profiles and microtubules.

Occasionally, nascent P-protein and ribosomes are close to the sieve plate in differentiating tubes (Fig. 8).

Discussion

Our present observations were undertaken with the purpose of completing our previous study in some Rutaceae on sieve-element plastids (Matarese Palmieri and Tomasello, 1983-1984).

The occurence of P-protein was briefly reported and commented; in fact in *Calodendrum capensis* the P-protein is of fibrillar type, in *Citrus limon* of fibrillar type, in *R. chalepensis* of fibrillar, tubular and crystalline type, but the origin was not discussed.

During early stages of development, either the sieve-elements, the companion cells, or the near parenchimatous cells, show nascent, tubular and crystalline forms of P-protein. The P-protein described by Esau and Cronshaw (1967) was studied as an amorphous, fibrillar, granular, tubular and crystalline type. During early stages of development of *Ruta,* the P-protein consists of fibrills associated with tubules as observed by Cronshaw and Esau (1968 a, b) and Esau (1971). We reached the same results as the above mentioned Authors; in fact the P-protein in at first of nascent type and finally of crystalline type.

Our study in *Ruta,* therefore, also agrees with Fjell's observations (1987), who observed the ontogeny of P-protein in *Salix viminalis:* the nascent and tubular P-protein. In some other Rutoideae we observed the tubular and fibrillar type P-protein but not the granular P-protein (Matarese Palmieri and Tomasello, 1983-1984). In some Aurantioideae we observed only the fibrillar P-protein because we did not investigate the ontogeny of P-protein (Matarese Palmieri and Tomasello, 1983-1984). According to Hoefert (1979-1980), coated vesicles are involved in the formation of granular P-protein. Cronshaw and Esau (1968 a, b) described the occurrence of vesicles with electron-dense material close

to the P-protein and considered them, as « spiny vesicles ». Also NEWCOMB (1967) observed the spiny vesicles during the formation of P-protein.

We described the occurrence of vesicles in some Rutaceae; now the present observations in *R. chalepensis* showed the vesicles close to dictyosomes with electron-dense material and closed to P-protein. On these basis, as in HOEFERT (1979) and CRONSHAW and ESAU (1968 a, b), the proximity of dictyosomes to the coated vesicles and to P-protein suggests that the P-protein takes origin from dictyosomes. The P-protein could rise from vesicles with a rupture followed by a discharge of their contents as interpreted by CRONSHAW and ESAU (1968 a, b). The spiny vesicles are closed to the nascent P-protein, while the coated vesicles seem to merge with P-protein bodies.

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SUMMARY

In this paper we report the characters of the P-protein in *Ruta chalepensis* L. We observed the nascent, tubular and crystalline type of P-protein. Also we observed the P-protein associated with microtubules, with spiny vesicles and dictyosomes.

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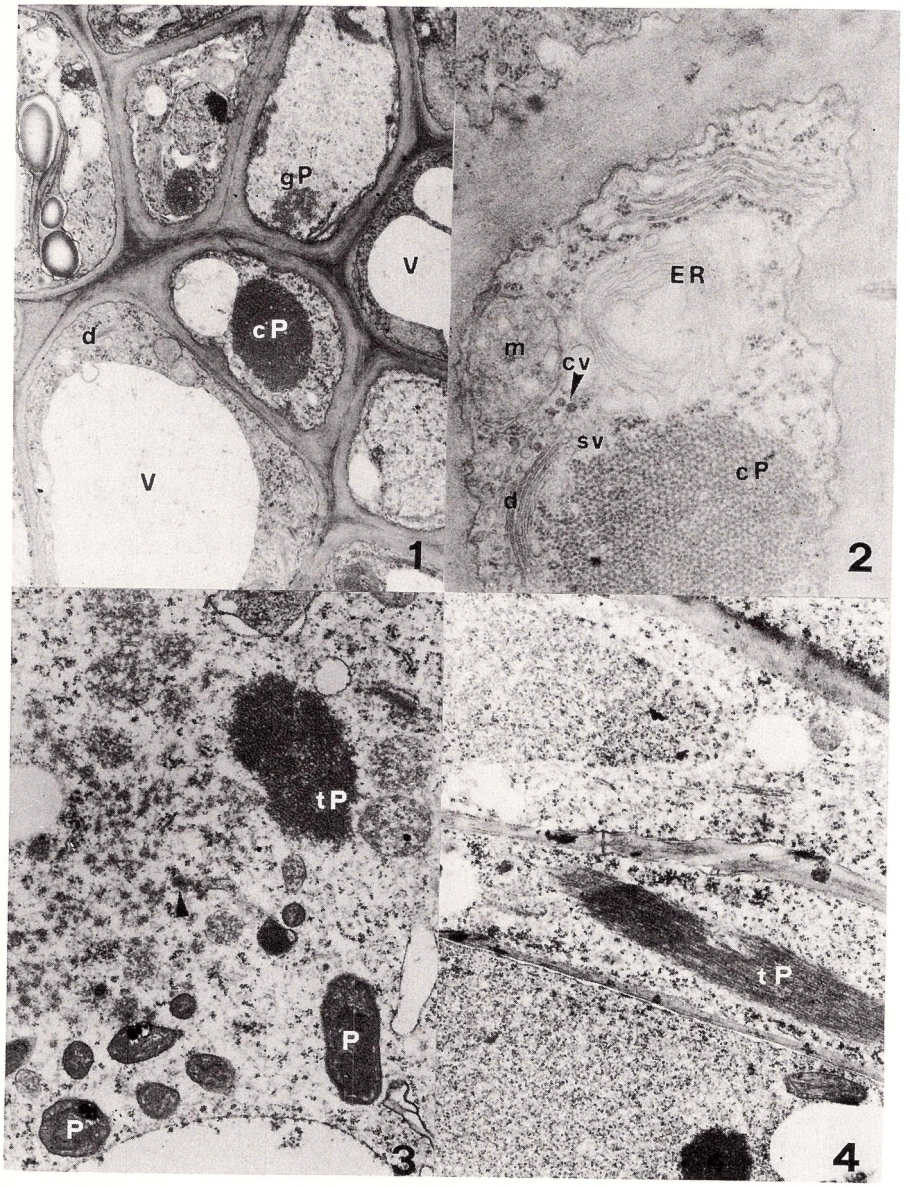
Differentiating sieve-elements of *R. chalepensis.*

Fig. 1. - Cross section of a leaf fixed in glutaraldehyde 4% for 2h. Differentiating phloem with dictyosomes (d), vacuoles (V), granular (gP) and crystalline (cP) P-protein. X27000.

Fig. 2. - Cross section of a leaf fixed in glutaraldehyde 6% for 4h. Note the changes of endoplasmic reticulum (ER), presence of mitochondria (m), coated vesicle (cv), spiny vesicles (sv), dictyosomes (d), polisomes and the P-protein of crystalline-type (cP). X32000. (From Matarese Palmieri and Tomasello, 1983-1984).

Fig. 3. - Cross section of a leaf fixed in glutaraldehyde 6% for 4h. Presence of tubular (tP) and nascent (see arrow) P-protein, plastids (P). X18000.

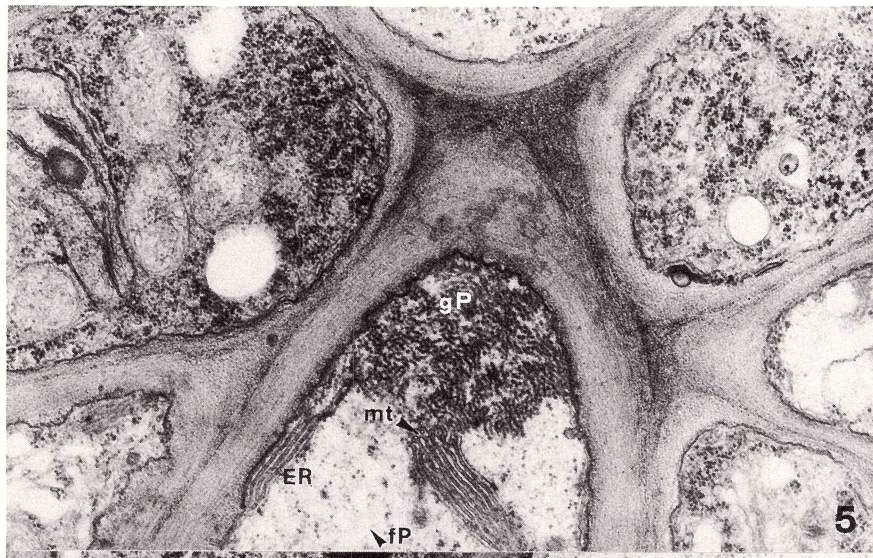
Fig. 4. - Longitudinal section of a leaf fixed in glutaraldehyde 6% for 4h with the Pprotein of tubular type (tP). X14000.

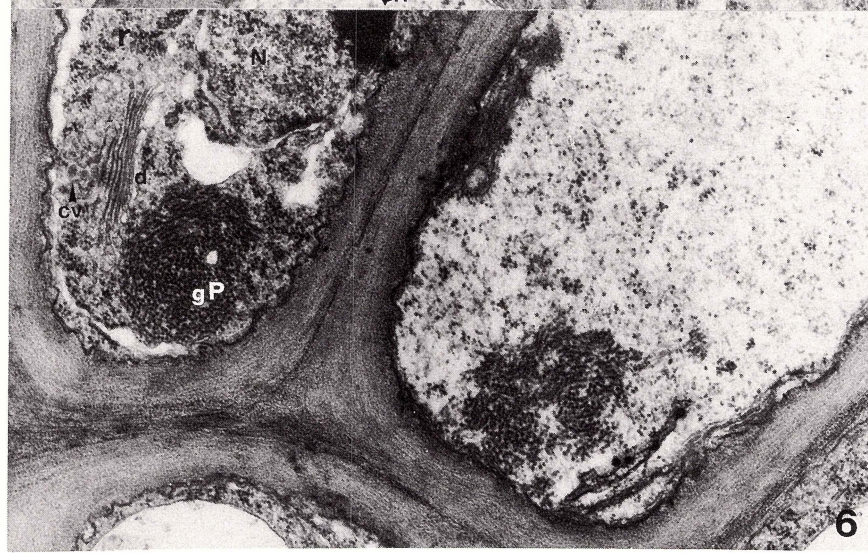

gP
V
d
cP
V
1
ER
m
cv
sv
cP
d
2
tP
P
P
3
tP
4

Differentiating sieve-elements of *R. chalepensis.*

Fig. 5. - Cross section of a leaf fixed in glutaraldehyde 4% for 2h. The P-protein granular (gP) associated with microtubules (mt), presence of fibrillar P-protein type (fP) and parietal endoplasmic reticulum (ER). X32000.

Fig. 6. - Cross section of a leaf fixed in glutaraldehyde 4% for 2h. Companion cell shows granular protein body (gP) coated vesicles (cv) dictyosomes (d), nucleus (N) and ribosomes (r). X33000.


gP
mt
ER
fP
5


r
N
d
cv
gP
6

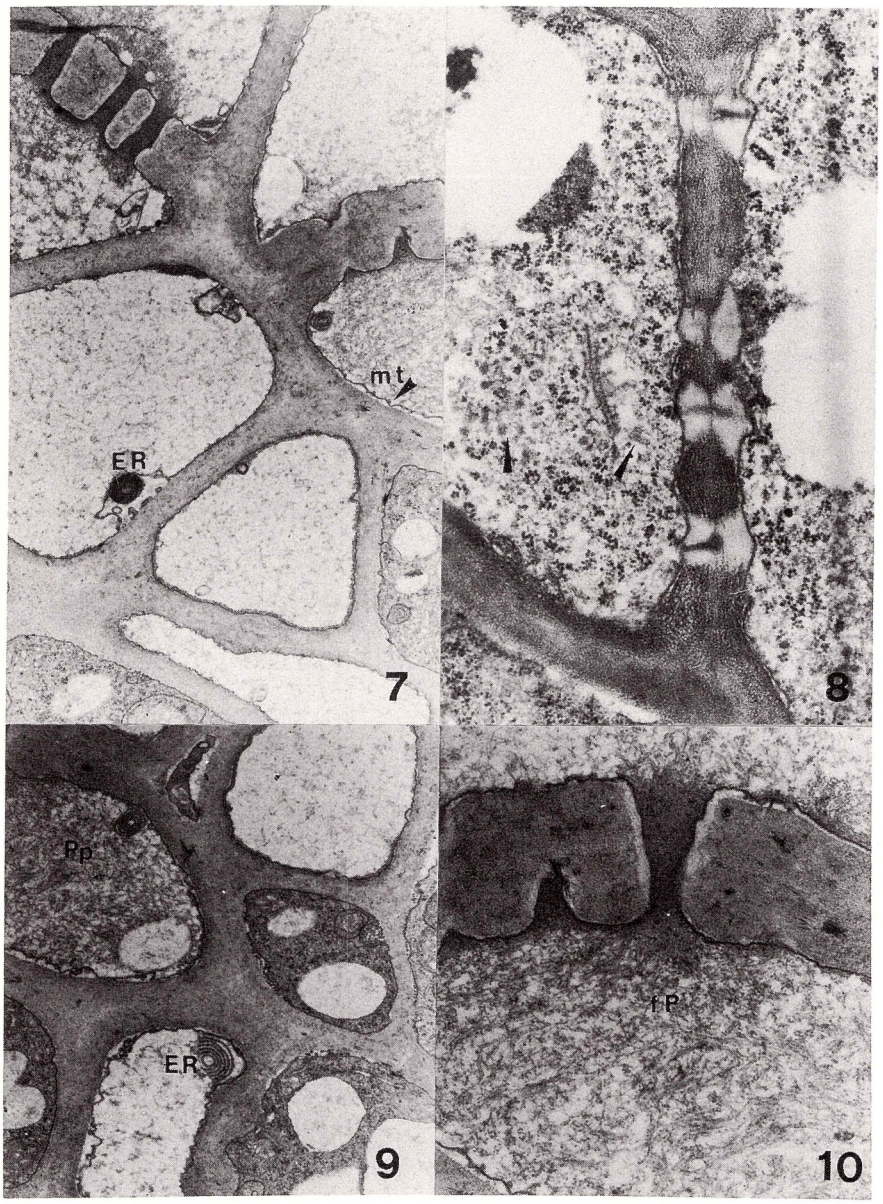
Differentiating and mature sieve-tube.

Fig. 7. - Cross section of a leaf fixed in glutaraldehyde 4% for 2h. Presence in mature sieve-tube of ER circular profiles, and microtubules (mt). X18000.

Fig. 8. - Section of leaf fixed in glutaraldehyde 6% for 2h with the sieve plate differentiating, note the nascent P-protein (see arrow). X18000.

Fig. 9. - Leaf fixed in glutaraldehyde 4% for 2h. Observe the circular profiles of ER and the P-protein in sieve tube (Pp). X18000.

Fig. 10. - Leaf fixed in glutaraldehyde 4% for 2h. Note fine filaments of P-protein in sieve plate (fP). X36000.


mt
ER
7
8
Pp
ER
9
fP
10